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## **RANK Ligand Blockade with Denosumab in Combination with Sorafenib in Chemorefractory Osteosarcoma: A Possible Step Forward?**

Cathomas, Richard ; Rothermundt, Christian ; Bode, Beata ; Fuchs, Bruno ; von Moos, Roger ;  
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**Abstract:** Background: There is no established systemic treatment option for unresectable osteosarcoma progressing after standard chemotherapy. A recently published clinical trial has demonstrated some activity of sorafenib in this situation. Preclinical research suggests a role for the inhibition of the receptor activator of nuclear factor- $\kappa$ B ligand (RANKL), but no clinical data have been reported so far. **Case Report:** A 37-year-old man was diagnosed with unresectable osteoblastic, osteoblastoma-like osteosarcoma in the C7/Th1 vertebra. The tumour progressed locally despite two lines of chemotherapy and stereotactic radiotherapy. On treatment with sorafenib and denosumab, a complete metabolic remission was achieved and is ongoing for over 18 months. Immunohistochemistry revealed an overexpression of RANK and RANKL in the patient's primary tumour. **Discussion:** This is the first report of activity achieved by the combination of the tyrosine kinase inhibitor sorafenib and the RANKL inhibitor denosumab in a patient with osteosarcoma. It confirms preclinical data on RANK/RANKL inhibition in osteosarcoma and could serve as a hypothesis-generating approach for clinical trials in this patient population. © 2014 S. Karger AG, Basel.

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# RANK Ligand Blockade with Denosumab in Combination with Sorafenib in Chemorefractory Osteosarcoma: A Possible Step Forward?

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## Key Words

Denosumab · Sorafenib · RANK · RANK ligand · Osteosarcoma

## Abstract

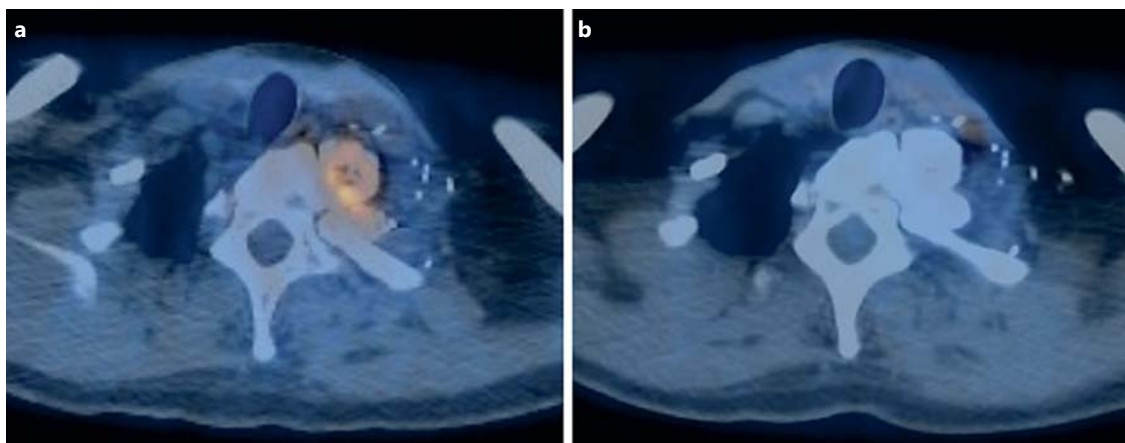
**Background:** There is no established systemic treatment option for unresectable osteosarcoma progressing after standard chemotherapy. A recently published clinical trial has demonstrated some activity of sorafenib in this situation. Preclinical research suggests a role for the inhibition of the receptor activator of nuclear factor- $\kappa$ B ligand (RANKL), but no clinical data have been reported so far. **Case Report:** A 37-year-old man was diagnosed with unresectable osteoblastic, osteoblastoma-like osteosarcoma in the C7/Th1 vertebra. The tumour progressed locally despite two lines of chemotherapy and stereotactic radiotherapy. On treatment with sorafenib and denosumab, a complete metabolic remission was achieved and is ongoing for over 18 months. Immunohistochemistry revealed an overexpression of RANK and RANKL in the patient's primary tumour. **Discussion:** This is the first report of activity achieved by the combination of the tyrosine kinase inhibitor sorafenib and the RANKL inhibitor denosumab in a patient with osteosarcoma. It confirms

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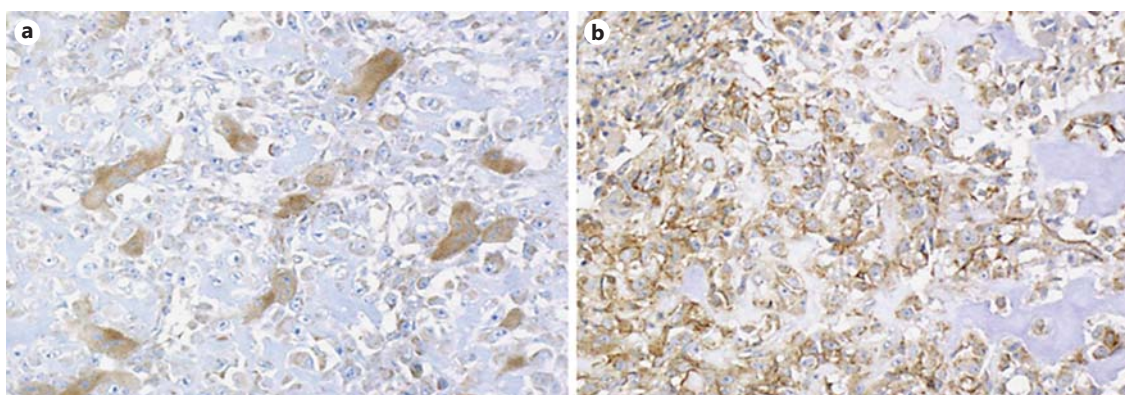
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## Introduction

Osteosarcoma is a rare primary malignant tumour of bone that can often be cured with multimodal approaches including combination chemotherapy and surgery [1]. Patients with primary osteosarcoma in the spine as well as male and older patients (aged >12 years) have an inferior prognosis [2, 3]. Treatment options are very limited in patients with inoperable locally advanced or metastatic disease that has progressed after chemotherapy or radiotherapy, and the outcome is poor [4]. A recently published phase II trial has suggested some benefit from treatment with the multi-target tyrosine kinase inhibitor sorafenib resulting in a clinical benefit rate of 29% (defined as improvement in the Pain Analgesic Score via the Brief Pain Inventory score) [5]. However, the benefit was short-lived, with a median progression-free survival of 4 months and a median overall survival of 7 months. The



**Fig. 1.**  $^{18}\text{F}$ -fluorodeoxyglucose positron emission tomography CT before (a) and 8 months after (b) the initiation of sorafenib/denosumab.



**Fig. 2.** a Immunohistochemistry of RANK (clone 80707, dilution 1:400; R&D Systems) demonstrating cytoplasmatic positivity in the multinucleated giant cells.  $\times 200$ . b Immunohistochemistry of RANKL (clone 12A668, dilution 1:400; Abcam) showing cytoplasmatic expression in the mononuclear, osteoid-producing tumour cells.  $\times 200$ .

receptor activator of nuclear factor- $\kappa\text{B}$  (RANK) signalling pathway is activated by RANK ligand (RANKL) and is essential for bone homeostasis [6]. Osteoblasts secrete RANKL, which leads to the differentiation and activation of osteoclasts promoting the release of bone-derived growth factors and osteolysis [6]. RANK signalling has been associated in preclinical osteosarcoma models with increased cell mobility and anchorage-independent growth [7]. In vitro studies in osteosarcoma cell lines using either osteoprotegerin (a physiological antagonist of RANK) or RANK-Fc protein to inhibit RANK signalling resulted in a reduction in osteosarcoma cell migration, invasion ability and anchorage-independent viability [8, 9]. In mice models, a reduction in tumour incidence, local

tumour growth and dissemination of lung metastases translating into prolonged survival of mice could be demonstrated by the same methods [10, 11]. Therefore, RANKL may represent a potential therapeutic target in osteosarcoma; however, so far, no clinical data using RANKL inhibition have been reported.

### Case Presentation

A 37-year-old otherwise healthy man was initially diagnosed histologically with an osteoblastoma in the Th1 vertebra. Curettage was performed, but the lesion recurred within 6 months in Th1 and C7. A pathology review revealed the diagnosis of an osteoblastic, osteoblastoma-like osteosarcoma. The tumour was not

primarily resectable in toto, and, after interdisciplinary discussion, the patient was treated with induction chemotherapy (cisplatin, doxorubicin, high-dose methotrexate) in analogy to the EURAMOS protocol [12]. After 3 cycles, local progression was noted, and the tumour remained unresectable, mainly because the patient was afraid of the potential surgical consequences. Instead, stereotactic radiotherapy (total dose: 80 Gy) was administered with little effect, and, after 2 months, further local progression was observed, and a new lesion at the resection margin of rib 1 was found. The patient received 3 cycles of second-line chemotherapy with ifosfamide, carboplatin and etoposide without any effect. At this point in time, treatment with sorafenib (400 mg twice daily) and denosumab (120 mg every 4 weeks) was initiated. The treatment has been well tolerated, with grade 2 hand-foot syndrome as the main side effect. A metabolic complete remission with intense calcifications at the areas of former  $^{18}\text{F}$ -fluorodeoxyglucose uptake (C7, Th1, rib 1) was noted in a positron emission tomography CT 8 months after initiation of treatment, with ongoing response and lack of further metastases after currently 18 months of treatment (fig. 1). Immunohistochemistry has been performed on formalin-fixed tissue of the patient's primary tumour, demonstrating positivity for RANK in multinucleated giant cells and cytoplasmatic expression of RANKL in the mononuclear, osteoid-producing tumour cells (fig. 2).

## Discussion

RANK and RANKL have been shown to be overexpressed in osteosarcoma patients and appear to be related to inferior outcome [13, 14]. In a retrospective study on 91 patients, overexpression of RANK and RANKL by immunohistochemistry was found in 69 and 9%, respectively, and RANK was associated with inferior disease-free survival [13]. Another retrospective study included 40 patients, and positivity for RANK and RANKL was found in 50 and 75%, respectively, and RANKL was related to a poor response to chemotherapy and inferior overall survival [14]. Denosumab is a fully human monoclonal an-

tibody against RANKL and effectively blocks the interaction between RANKL and RANK, leading to decreased bone resorption [15]. Denosumab is well tolerated and used in the treatment of osteoporosis as well as in patients with bone metastases from solid tumour, reducing the number of skeletal-related events [16]. It has also shown very encouraging activity in giant cell tumour of bone, a locally aggressive primary bone tumour in which RANKL plays a critical role in pathogenesis [17], and this led to the approval of denosumab for the treatment of giant cell tumour by the European Medicines Agency. In vitro studies show that RANK activation initiates multiple signalling pathways beyond the usual target of nuclear factor- $\kappa\text{B}$ . Especially the ERK1/2 pathway has been found to be crucial for the promotion of anchorage-independent survival and invasion of osteoblastic cells [7]. This is even more interesting since it appears that a blockade of the ERK1/2 pathway might be one of the mechanisms of action of sorafenib in osteosarcoma [18, 19]. A synergistic effect of the combination of denosumab and sorafenib can therefore be proposed. No preclinical data have been published investigating this hypothesis.

To our knowledge, no reports on the treatment with denosumab or the combination of sorafenib and denosumab in the setting of osteosarcoma have been published so far. The exceptional response in our patient and the outlined preclinical evidence might serve as hypothesis generating and trigger an interest in prospective trials.

## Disclosure Statement

Richard Cathomas: advisory role for Bayer. Roger von Moos: advisory role for Amgen. All remaining authors have declared no conflicts of interest.

## References

- Anninga JK, Gelderblom H, Fiocco M, et al: Chemotherapeutic adjuvant treatment for osteosarcoma: where do we stand? *Eur J Cancer* 2011;47:2431–2445.
- Schoenfeld AJ, Hornicek FJ, Pedlow FX, et al: Osteosarcoma of the spine: experience in 26 patients treated at the Massachusetts General Hospital. *Spine J* 2010;10:708–714.
- Collins M, Wilhelm M, Conyers R, et al: Benefits and adverse events in younger versus older patients receiving neoadjuvant chemotherapy for osteosarcoma: findings from a meta-analysis. *J Clin Oncol* 2013;31:2303–2312.
- Bielack SS, Kempf-Bielack B, Branscheid D, et al: Second and subsequent recurrences of osteosarcoma: presentation, treatment, and outcomes of 249 consecutive cooperative osteosarcoma study group patients. *J Clin Oncol* 2009;27:557–565.
- Grignani G, Palmerini E, Dileo P, et al: A phase II trial of sorafenib in relapsed and unresectable high-grade osteosarcoma after failure of standard multimodal therapy: an Italian Sarcoma Group study. *Ann Oncol* 2012;23:508–516.
- Dougall WC, Glaccum M, Charrier K, et al: RANK is essential for osteoclast and lymph node development. *Genes Dev* 1999;13:2412–2424.
- Beristain AG, Narala SR, Di Grappa MA, Khokha R: Homotypic RANK signaling differentially regulates proliferation, motility and cell survival in osteosarcoma and mammary epithelial cells. *J Cell Sci* 2012;125:943–955.
- Lamoureaux F, Richard P, Wittrand Y, et al: Therapeutic relevance of osteoprotegerin gene therapy in osteosarcoma: blockade of the vicious cycle between tumor cell proliferation and bone resorption. *Cancer Res* 2007;67:7308–7318.

- 9 Akiyama T, Choong PF, Dass CR: RANK-Fc inhibits malignancy via inhibiting ERK activation and evoking caspase-3-mediated anoikis in human osteosarcoma cells. *Clin Exp Metastasis* 2010;27:207–215.
- 10 Lamoureux F, Picarda G, Rousseau J, et al: Therapeutic efficacy of soluble receptor activator of nuclear factor- $\kappa$ B-Fc delivered by nonviral gene transfer in a mouse model of osteolytic osteosarcoma. *Mol Cancer Ther* 2008;7:3389–3398.
- 11 Akiyama T, Dass CR, Shinoda Y, et al: Systemic RANK-Fc protein therapy is efficacious against primary osteosarcoma in a murine model via activity against osteoclasts. *J Pharm Pharmacol* 2010;62:470–476.
- 12 EURAMOS 1: A randomized trial of the European and American Osteosarcoma Study Group to optimize treatment strategies for resectable osteosarcoma based on histological response to pre-operative chemotherapy (IS-RCTN67613327).
- 13 Bago-Horvath Y, Schmid K, Rosseler F, et al: Impact of RANK signaling on survival and chemotherapy response in osteosarcoma. *Pathology* 2014;46:411–415.
- 14 Lee JA, Jung JS, Kim DH, et al: RANKL expression is related to treatment outcome of patients with localized, high-grade osteosarcoma. *Pediatr Blood Cancer* 2011;56:738–743.
- 15 Lacey DL, Boyle WJ, Simonet WS, et al: Bench to bedside: elucidation of the OPG-RANK-RANKL pathway and the development of denosumab. *Nat Rev Drug Discov* 2012;11:401–419.
- 16 Peddi P, Lopez-Olivo MA, Pratt GF, et al: Denosumab in patients with cancer and skeletal metastases: a systematic review and meta-analysis. *Cancer Treat Rev* 2013;39:97–104.
- 17 Chawla S, Henshaw R, Seeger L: Safety and efficacy of denosumab for adults and skeletally mature adolescents with giant cell tumour of bone: interim analysis of an open-label, parallel-group, phase 2 study. *Lancet Oncol* 2013;14:901–908.
- 18 Pignochino Y, Grignani G, Cavalloni G, et al: Sorafenib blocks tumour growth, angiogenesis and metastatic potential in preclinical models of osteosarcoma through a mechanism potentially involving the inhibition of ERK1/2, MCL-1 and ezrin pathways. *Mol Cancer* 2009;8:118.
- 19 Mei J, Zhu X, Wang Y, Wang Z: VEGFR, RET and RAF/MEK/ERK pathway take part in the inhibition of osteosarcoma MG63 cells with sorafenib treatment. *Cell Biochem Biophys* 2014;69:151–156.